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Nitrogen Deficiency-Dependent Abiotic Stress Enhances Carotenoid Production in Indigenous Green Microalga *Scenedesmus rubescens* KNUA042, for Use as a Potential Resource of High Value Products

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Table S1. Results from BLAST searches using the sequences of 18S rRNA, ITS2, and *rbcL* genes of strain KNUA042.

Marker gene	Accession no.	Product size (bp)	Closet match (GenBank accession no.)	Overlap (%)	Sequence similarity (%)
18S rRNA	MT645779	1,767	*Scenedesmus rubescens* KMMCC 263 (JQ315585)	99%	99.49%
ITS 2	MT645778	426	*Scenedesmus* sp. SM8_2 (KT778097)	100%	99.53%
rbcL	MT655944	1,385	*Acutodesmus deserticola* BCP-YPG-Char (HQ246361)	94%	98.86%

Figure S1. (**a**) Map of the study area, located on the East sea, Korea. (**b**) Sampling location of *S. rubescens* in the main islands of South Korea. (**c**) Light microscopy of *S. rubescens* KNUA042.

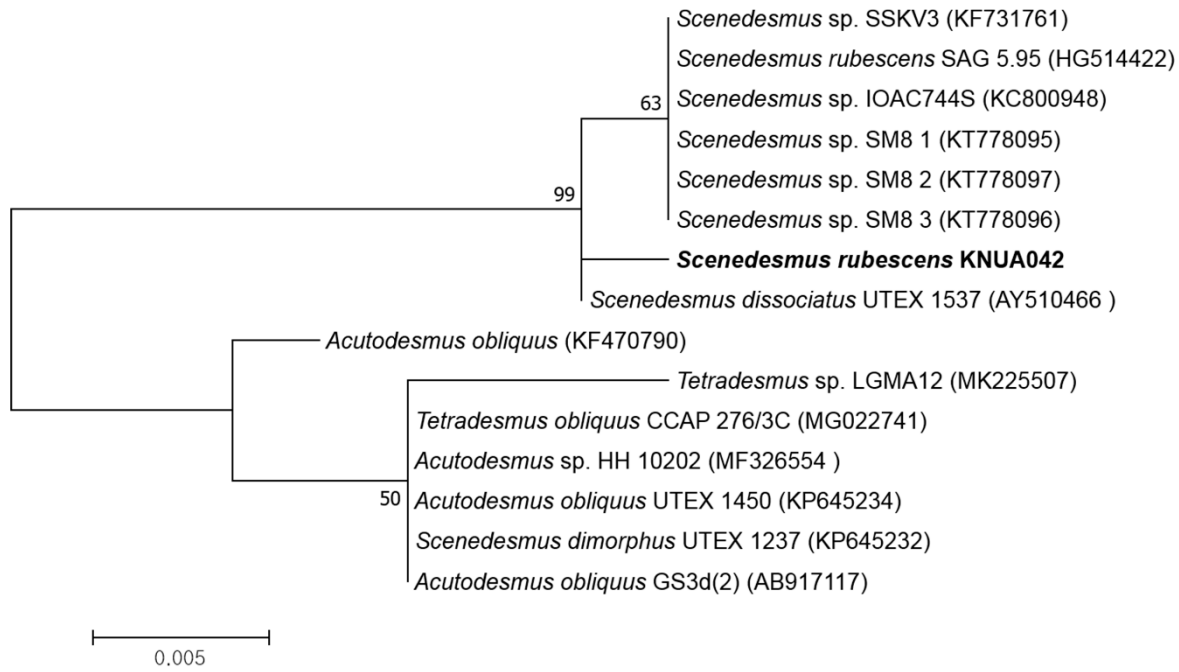

Figure S2. Phylogenetic analysis of KNUA042 and its closely related species based on ITS2 region sequences. Numbers in parentheses are accession numbers for each sequence in GenBank. The tree was generated by the ML method using bootstrap values with 1,000 replicates. The scale bar measures the distance between species.

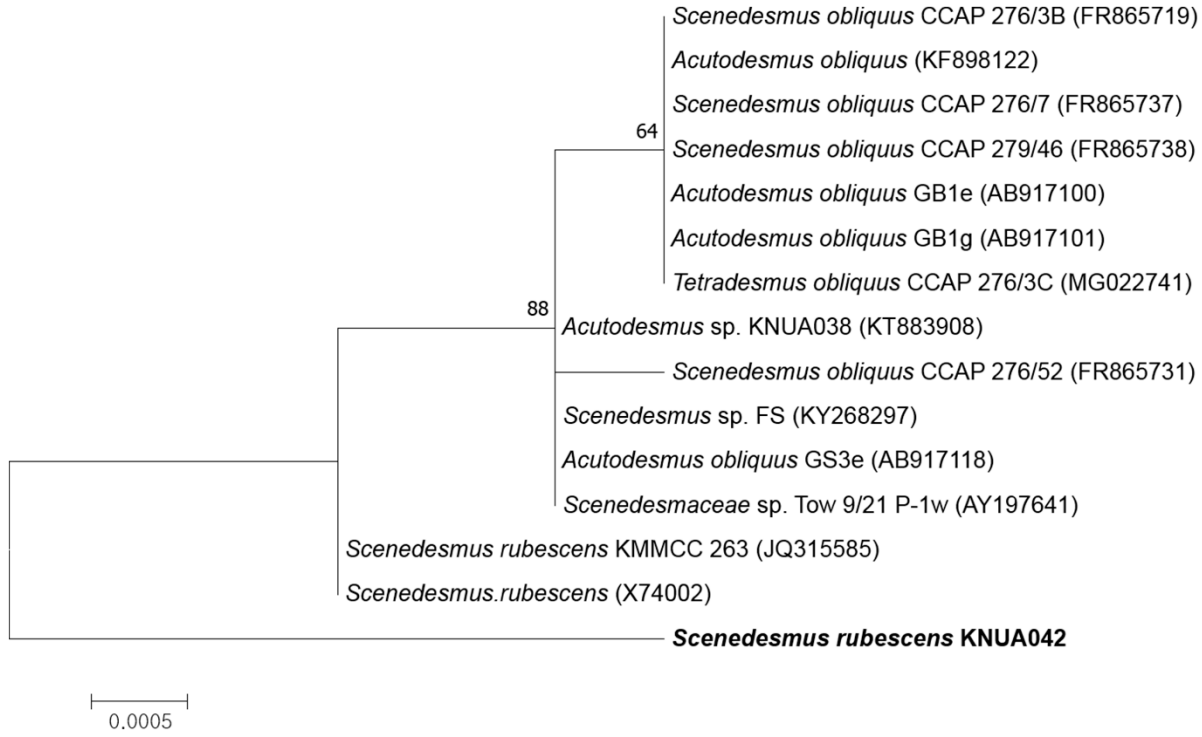

Figure S3. Phylogenetic analysis of KNUA042 and closely related species based on 18S rRNA sequences. Numbers in parentheses are accession numbers for each sequence in GenBank. The tree was generated by the ML method using bootstrap values with 1,000 replicates. The scale bar measures the distance between species.

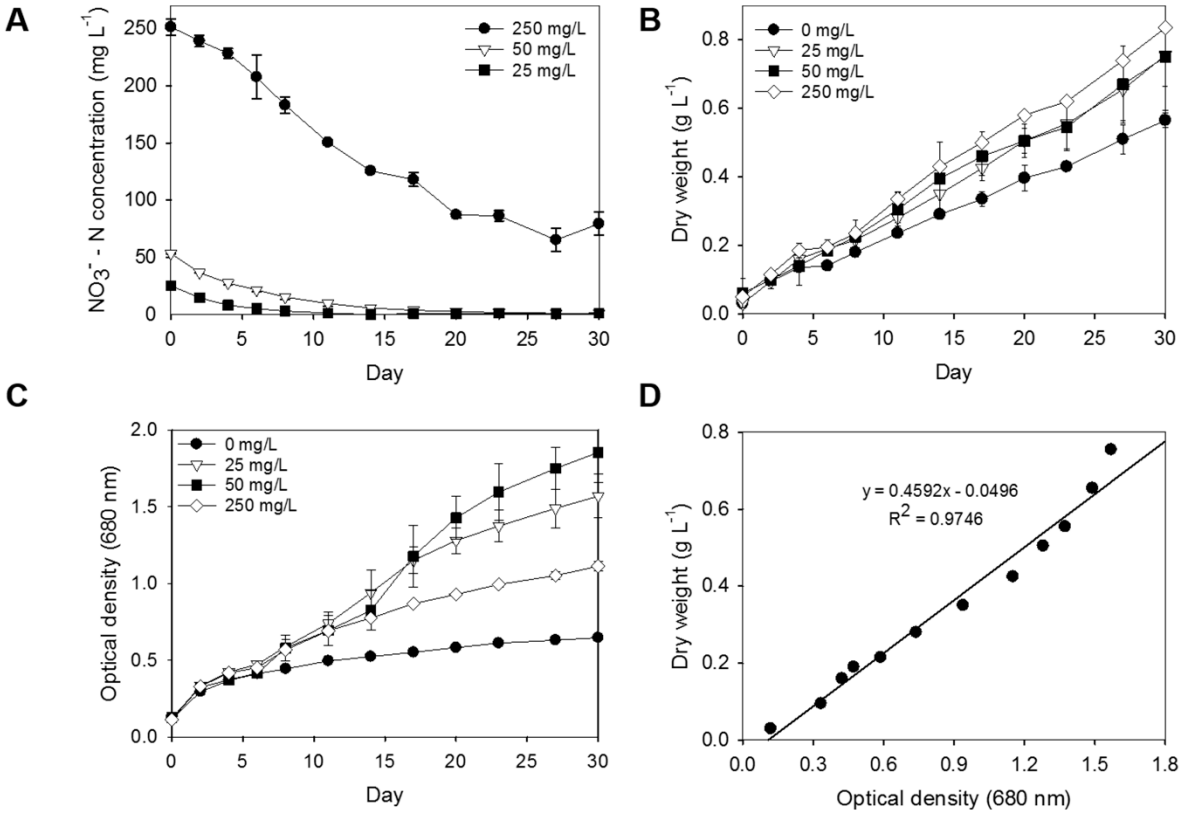

Figure S4. (**a**) Comparison of nitrate-N concentration, (**b**) dry weight, and (**c**) optical density from *S. rubescens* KNUA042 cells under different nitrate-N concentrations. (**d**) Correlation between biomass density and optical density for *S. rubescens* cultivation. The error bar represents the standard deviation (SD) of biological replicates and are shown as mean ± SD, n = 3.

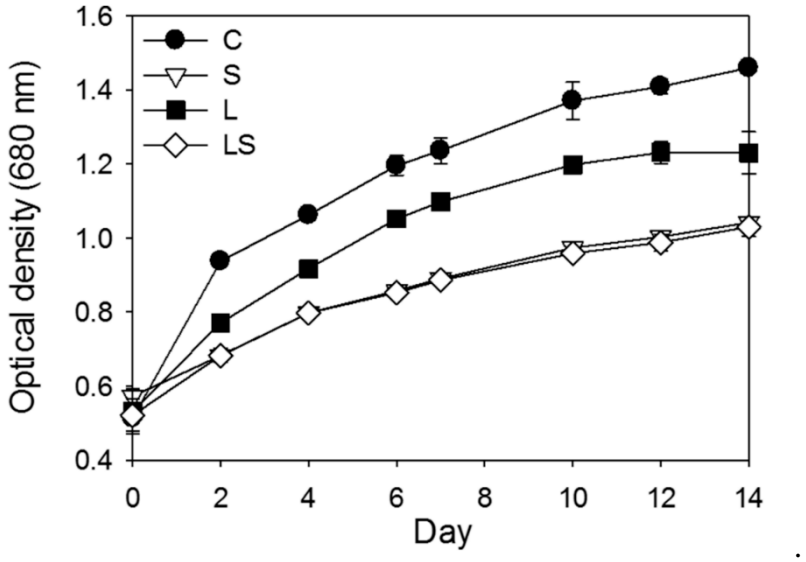

Figure S5. Growth rate of *S. rubescens* KNUA042 cells under various stresses from day 0 to day 14. The error bar represents the standard deviation (SD) of biological replicates and are shown as mean ± SD, n = 3.

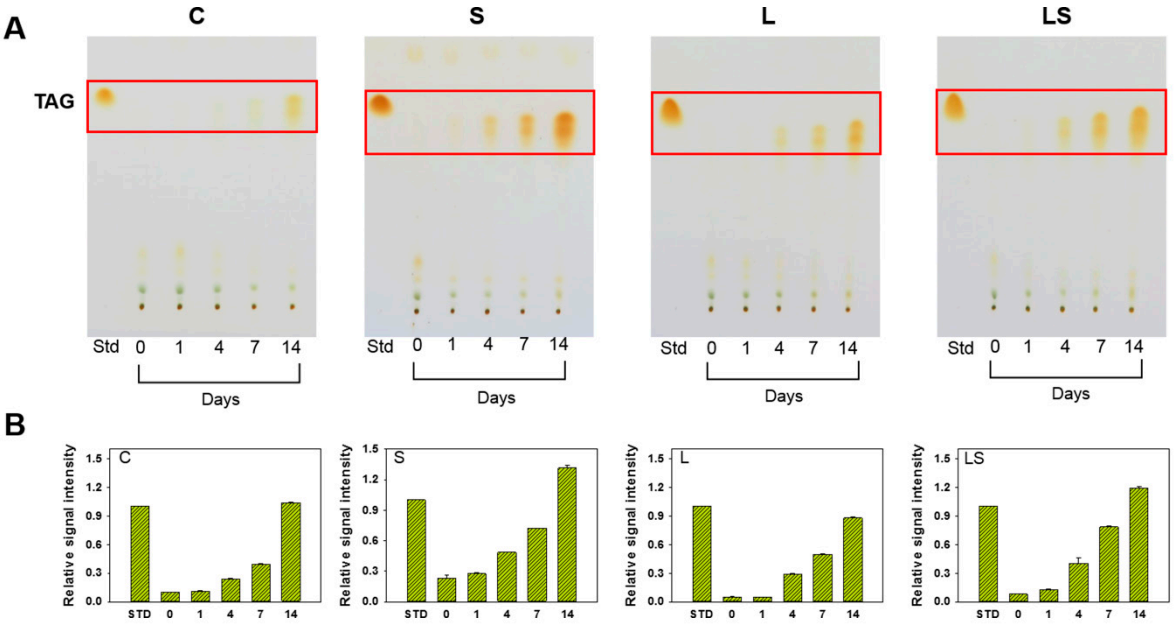

Figure S6. (**a**) Thin layer chromatographic (TLC) analysis of total lipids in *S. rubescens* KNUA042 cells under different stress conditions on day 14 of cultivation. (**b**) The average relative intensity of TAG with different stress types was determined using ImageJ. The error bar represents the standard deviation (SD) of biological replicates and are shown as mean ± SD, n = 3. TAG, triacylglycerols; Std, glyceryl trioleate.

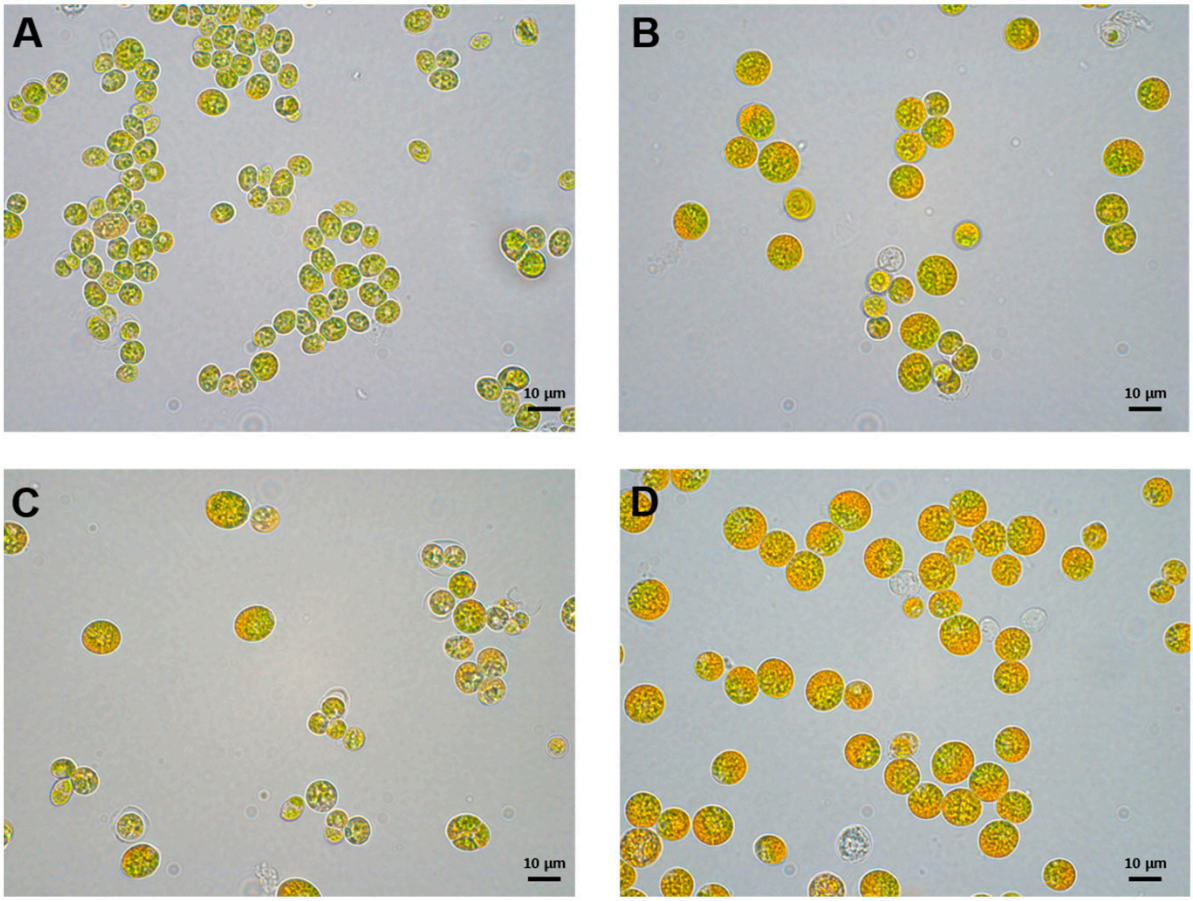

Figure S7. Microscopic image of stressed *S. rubescens* KNUA042 cells subjected to (**a**) nitrogen deficiency, (**b**) salinity, (**c**) high light intensity, and (**d**) combined, high light intensity + salinity) during the final days of cultivation. Scale bar 10 µm.

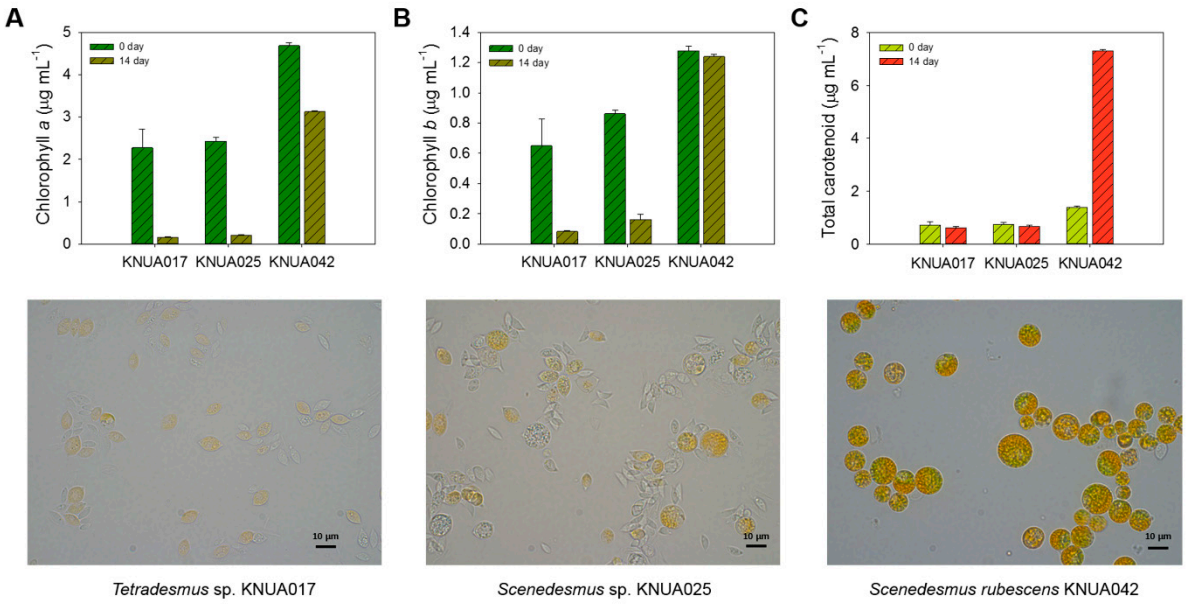

Figure S8. Comparison of (**a**) chlorophyll *a* content, (**b**) chlorophyll *b*, (**c**) total carotenoid content in different microalgae strains. The error bar represents the standard deviation (SD) of biological replicates and are shown as mean ± SD, n = 3.

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